MORPHOLOGY, YIELD AND BREEDING BEHAVIOR OF DIPLOID *DENDROBIUM* SPECIES HYBRIDS AND THEIR CORRESPONDING AMPHIDIPLOIDS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

MAY 1991

By

Teresita D. Amore

Dissertation Committee:

Haruyuki Kamemoto, Chairman
Gerald D. Carr
Richard W. Hartmann
Charles L. Murdoch
James A. Silva

We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Horticulture.

DISSERTATION COMMITTEE

Chairman

ABSTRACT

Morphology, yield and breeding behavior of eleven diploid Dendrobium species hybrids and their corresponding colchicine-induced amphidiploids from intersectional combinations of Ceratobium x Eleutheroglossum, Phalaenanthe x Ceratobium and Phalaenanthe x Eleutheroglossum were compared. Increased flower width and broader petals and sepals resulted in increased flower size of the amphidiploids. The amphidiploids were less floriferous and produced fewer and shorter racemes than their corresponding diploids, although statistical significance varied from selection to selection.

Irregular sporad formation was observed in two Ceratobium-Eleutheroglossum amphidiploid selections, while a preponderance of tetrads was produced by the Phalaenanthe-Ceratobium amphidiploid selections. The Phalaenanthe-Eleutheroglossum diploid selection produced more tetrads than its amphidiploid counterpart.

Fertility was evaluated by crossing the diploids and the amphidiploids with D. rumphianum and D. stratiotes. Cross combinations with the diploid selections resulted in low fruit set and low embryo viability. Male sterility was observed in the majority

of the crosses, although female fertility increased with chromosome doubling.

D. xJaquelyn Thomas 'K333-22', D. xMemoria Edward Trevor 'D251', D. superbiens 'D184' and D. xAutumn Lace 'K432-2' were evaluated for their potential use as parents for seed-propagated cultivars. Offspring of the amphidiploids D. xJaquelyn Thomas 'K333-22', D. xMemoria Edward Trevor 'D251' and D. xAutumn Lace 'K432-2' were variable, and were therefore not desirable parents for the production of seed propagated potted plant cultivars. Amphidiploid D. superbiens 'D184' crossed with D. phalaenopsis produced uniform D. xLouis Bleriot progeny which can be used for lei flower production.

Eleven crosses were made in 1985 using three clones of D. xLouis Bleriot as female parents and three clones of amphidiploid D. xJaquelyn Thomas and one clone of D. xNeo-Hawaii as male parents to substantiate the occurrence of androgenesis. Chromosome numbers of the majority of hybrid offspring were aneuploid ranging between the triploid and tetraploid levels, which is expected of 3N x 4N crosses. Some diploids which strongly resembled the male parents were recovered in 10 out of the 11 crosses and were presumed to be androgenetic. Although the parent amphidiploids were

heavily infected with Cymbidium Mosaic Virus, the virus was not detected in most of the androgenetic diploids.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Dendrobium is the largest genus in the orchid family, comprising over 1000 species subdivided into 41 sections (Schlecter, 1912). Although the sections Phalaenanthe, Ceratobium, Eleutheroglossum, Latourea, Callista, Nigrohirsutae and Eugenanthe all have horticultural importance, (Kamemoto and Wilfret, 1980; Kamemoto, 1980), the first four sections are most important for the breeding of commercial cut flowers and potted flowering plants for the tropics (Kamemoto and McConnell, 1984).

The University of Hawaii has developed and released seed-propagated cultivars which have become the backbone of the dendrobium industry of Hawaii. The advantages of seed-propagated over clonally propagated cultivars are faster and cheaper propagation, and the production of seedlings free of Cymbidium Mosaic Virus (Yuen et al., 1979), since this virus is not transmitted through seed (Kamemoto and McConnell, 1984; Kamemoto, 1985).

The development of seed propagated cultivars has relied on amphidiploidy. Diploid species hybrids of the Phalaenanthe-Ceratobium combination often exhibit irregular meiosis resulting in impaired fertility (Kamemoto et al., 1964; Wilfret et al., 1979).

Doubling of chromosomes results in amphidiploids with normal pairing at meiosis and restored fertility (Kamemoto et al, 1964; McConnell, 1984).

The first five seed-propagated cut flower cultivars released by the University of Hawaii involved four Phalaenanthe-Ceratobium amphidiploids (Kamemoto, 1985). Recently, selections of intersectional diploid species hybrids of the Ceratobium-Eleutheroglossum, Phalaenanthe-Ceratobium and Phalaenanthe-Eleutheroglossum combinations were treated with colchicine in tissue culture (Sanguthai et al., 1973) in order to obtain additional amphidiploids for the breeding program. Several new amphidiploids have been produced, including those obtained from doubling polyhaploid offspring of the four Phalaenanthe-Ceratobium amphidiploids previously used in the breeding program. The objectives of this study were to evaluate the characteristics of these amphidiploids and their corresponding diploid counterparts, and to assess the breeding behavior of some of the newly obtained amphidiploids.

2. REVIEW OF LITERATURE

2.1 Polyploid Classification

Changes in chromosome number which either increase or decrease the number by a complete genome are termed euploidy. Polyploidy is a type of euploidy that occurs when an organism possesses three or more complete genomes (Jackson, 1976).

Stebbins (1947) recognized three classes of polyploids at the tetraploid level, namely auto-polyploids, segmental allopolyploids and true allopolyploids. Autopolyploids have similar component genomes and are derived from relatively homozygous diploids, or from hybrids between varieties or subspecies of a diploid species. Autopolyploids are characterized by multivalents at meiosis and tetrasomic ratios. In artificially produced autopolyploids, slower development and reduced fertility were observed. True allopolyploids are derived from different genomes and are characterized by bivalents at meiosis, although multivalent associations and tetrasomic ratios may rarely occur. These resemble diploids in their cytogenetic behavior. Segmental allopolyploids are derived from diploid hybrids which have good pairing at meiosis, but whose genomes differ sufficiently so that interchange between chromosome segments or gene

combinations is limited by partial or complete sterility at the diploid level.

2.2 Origin of Polyploid Orchids

One way polyploidy can develop is from unreduced gametes functioning in fertilization. Polyploid gametes arise by: a) recombination of second division products; b) failure of first division or recombination of the first division products followed by an equational second division; c) failure of first division followed by recombination of products of an equational second division; d) somatic doubling of the pre-meiotic sporocyte followed by assortment of the chromosomes; or e) somatic doubling of the pre-meiotic sporocyte without subsequent division or assortment (Storey, 1956).

Polyploids may arise from abnormalities such as fusion of a synergid and the egg, fertilization of the egg by more than one sperm (Hagerup, 1947), or from spontaneous doubling in tissue culture as observed in Dendrobium xJaquelyn Thomas 'O580' and in D. xNeo-Hawaii 'Y972', or in the diploid hybrid populations of D. xJaquelyn Thomas 'Y166-1' and D. xJaquelyn Thomas 'D168-12' (Kamemoto, 1980).

Another way polyploidy can develop is by artificial induction using colchicine. Early attempts

were made using seedlings or shoots, although success was not substantiated by chromosome counts (MacLeod, 1947; Rotor, 1958). Nakasone and Kamemoto (1961) treated seeds, protocorms, seedlings, inflorescences, cuttings, young shoots and apical meristems of mature plants with a low success rate. Menninger (1963) treated dormant backbulbs of Cymbidium with 0.3% colchicine, followed by 1% colchicine after 10 days to produce tetraploids. In vitro treatment of orchids was first reported by Wimber and Van Cott (1967) using 0.05% colchicine on Cymbidium seedlings and meristem-tissue cultures in agitated liquid culture. About 40% of either the treated seedlings or the meristem plantlets became tetraploid. Similar results have been reported for Dendrobium (Sanguthai, et al., 1973), Paphiopedilum (Watrous and Wimber, 1988), Phalaenopsis (Griesbach, 1985) and Vanda (Sanguthai and Sagawa, 1973).

2.3 Effects of Polyploidy on Plant Morphology

Polyploidy in orchids has been associated with horticultural superiority. An increase in ploidy often results in flowers with larger and more overlapping sepals and firm petals of heavier substance (Kosaki and Kamemoto, 1961). Colchicine-induced tetraploid

Cymbidium showed increased flower size (Wimber and Wimber, 1968). Similar results were reported for Cymbidium (Leonhardt, 1987); Aranda (Kam and Kamemoto, 1980) Dendrobium (Chaicharoen and Sajaew, 1981; McConnell, 1984), and Phalaenopsis (Griesbach, 1985).

A reduction in inflorescence length and number of flowers per inflorescence was reported for tetraploid Cymbidium (Wimber and Wimber, 1968; Leonhardt, 1987) and tetraploid Aranda xWendy Scott 'Greenfield' (Kam and Kamemoto, 1980). Longer and more erect racemes were observed in tetraploid Dendrobium xJaquelyn Thomas '0580' compared to the diploid form (Kamemoto, 1980). Racemes produced by tetraploid D. xNeo-Hawaii were frequently distorted and bud drop percentage was higher than that of the diploid.

Stomatal size was larger in tetraploid Vanda xMiss Joaquim (Nakasone and Kamemoto, 1961). However, the number of stomates was not changed. Guard cell size was larger in tetraploid Dendrobium phalaenopsis (Chaicharoen and Sajaew, 1981). Leaves of the tetraploid were also thicker.

Tetraploid Paphiopedilum plants developed faster in vitro than the diploids of the same age. Tetraploid plants were darker green, and had leaves with greater substance than their diploid counterparts (Watrous and Wimber, 1988).

2.4 Cytogenetics of Diploid and Polyploid Species Hybrids

Interspecific Vanda hybrids among diploid strap-leaved species (SS) or among terete-leaved species (TT), showed meiotic regularity and homology of parental chromosomes (Tanaka and Kamemoto, 1960). Diploid species hybrids between strap-leaved and terete-leaved species (ST) exhibited highly irregular meiosis and poor homology of parental chromosomes. SS or TT hybrids were fertile, while ST hybrids exhibited reduced fertility. Multivalents were occasionally observed in autotetraploids (SSSS or TTTT), while bivalents were predominantly produced in the allotetraploid (SSTT). Both the autotetraploid and the allotetraploid were found to be fertile.

Highly irregular meiosis was observed in diploid Aranda xWendy Scott 'Greenfield', a hybrid between Vanda xRothschildiana and Arachnis hookeriana, while the amphidiploid form had relatively regular meiosis (Lee and Kamemoto, 1984). Sporad types produced by the diploid ranged from dyads to octads with microcytes, while the amphidiploid produced predominantly tetrads.

In Dendrobium, the Phalaenanthe-Ceratobium diploid hybrid, D. xJaquelyn Thomas (D. phalaenopsis x D. gouldii), formed 13-19 bivalents at meiosis, while the amphidiploid formed 38 bivalents (Kamemoto et al.,

1964). Primary hybrids between Phalaenanthe and Ceratobium produced about 17 out of 19 possible chromosome pairs at meiosis and generally showed impaired fertility. Chromosome doubling of the species hybrids resulted in restored fertility (Kamemoto and Wilfret, 1980; Wilfret et al., 1979).

McConnell (1984) investigated meiosis and sporad formation in three interspecific Ceratobium-Phalaenanthe hybrids. The diploids showed irregular meiosis with mean configurations ranging from 14.3 bivalents and 9.4 univalents to 18.9 bivalents and 0.1 univalents. The amphidiploid counterparts showed regular meiosis with 38 bivalents. The diploids produced 36 to 70% tetrads, while the amphidiploids produced 97 to 100% tetrads.

2.5 Breeding Behavior of Amphidiploid Dendrobium

Selfing the Phalaenanthe-Ceratobium amphidiploids of D. xJaquelyn Thomas 'Y166-1' (Bobisud and Kamemoto, 1982), D. xJaquelyn Thomas '0580' and D. xNeo Hawaii 'Y972' (McConnell, 1984) resulted in relatively uniform progeny, indicative of preferential pairing within genomes. Crossing the Phalaenanthe-Ceratobium amphidiploids resulted in uniform progeny with desirable traits, and led to the naming and release of

seed-propagated cultivars for cutflower production (Kamemoto and McConnell, 1984; Kamemoto, 1985).

2.6 Androgenesis in Dendrobium

Androgenesis is the development of a haploid individual with only the paternal chromosome complement. In contrast, gynogenesis is the development of a haploid individual without syngamy (Rieger, Michaelis and Green, 1976). Androgenesis occurs when the maternal nucleus is eliminated or inactivated subsequent to fertilization of the egg cell. Pandey (1973) preferred the term "ovule androgenesis" for haploid forms derived from the development of the male gamete in the embryo sac of an ovule, in contrast with "anther androgenesis", in which there is no participation of female cytoplasm (e.g., haploids derived from the immature anthers of plants).

Although androgenesis is rare, its occurrence has been reported in Capsicum frutescens (Campos and Morgan, 1958), maize (Goodsell, 1961; Chase, 1963), tobacco (Burk, 1962), begonia (Horn, 1977) and cacao (Lanaud, 1988).

In Dendrobium, androgenesis was observed in progeny of 'Louis Bleriot'. 'Louis Bleriot' is a triploid hybrid of D. superbiens (Phalaenanthe-

Ceratobium) and D. phalaenopsis (Phalaenanthe). When crossed with D. xJaquelyn Thomas, progeny with the haploid chromosome number and morphology of the male parent were recovered and were determined to be androgenetic.

3. MATERIALS AND METHODS

3.1 Morphology and Yield Comparisons in Diploid Species Hybrids and Their Corresponding Amphidiploids

3.1.1 Plant Material

Eleven diploid intersectional species hybrids and their corresponding amphidiploids were available for evaluation (Table 3.1). The intersectional hybrids are combinations of Ceratobium-Eleutheroglossum, Phalaenanthe-Ceratobium and Phalaenanthe-Eleutheroglossum. Individual plants selected from intersectional crosses are preceded by "K", while single plant accessions are preceded by "D". Eleutheroglossum is represented by the miniature species D. canaliculatum. Dockrill (1969) in his "Australian Indigenous Orchids" placed this species in the section Eleutheroglossum. More recently Cribb (1986) included it in the section Ceratobium (which was renamed Spatulata on grounds of priority). However, because the genome E was assigned for D. canaliculatum following Dockrill's earlier classification (Kamemoto, 1980; Kamemoto and Wilfret, 1980), the section Eleutheroglossum has been retained for this study.

The selections from diploid species crosses or accessions were put in tissue culture for clonal multiplication. Amphidiploids were earlier produced by treating protocorm-like bodies of the selections

Table 3.1. Designation used for intersectional *Dendrobium* species hybrids for which diploids and corresponding amphidiploids were evaluated (*C*=*Ceratobium*, *E*=*Eleutheroglossum* and *P*=*Phalaenanthe*).

Hybrid Selection	Parentage	Intersectional Combination	Registered Name
K227-27	*antennatum* 'SL-1' x *canaliculatum* 'D129'	*C* x *E*	Lowana Nioka
K228-21	*tokai* x *canaliculatum*	*C* x *E*	Tocanal
K432-2	*canaliculatum* x *strebloceras*	*E* x *C*	Autumn Lace
K333-22	*gouldii* 'D158' x *phalaenopsis* 'H2-4'	*C* x *P*	Jaquelyn Thomas
K461-22	gynogenetic selection: Jaquelyn Thomas 'Y166-1 4N' x *superbiens* '4N'	*P* x *C*	Jaquelyn Thomas
K522-1	androgenetic selection: Louis Bleriot 'LBH' x Jaquelyn Thomas 'O580 4N'	*P* x *C*	Jaquelyn Thomas
K523-4	androgenetic selection: Louis Bleriot 'LBH' x Neo Hawaii 'Y972 4N'	*P* x *C*	Neo Hawaii
K524-135	androgenetic selection: Louis Bleriot 'LBU' x Jaquelyn Thomas 'D168-12'	*P* x *C*	Jaquelyn Thomas
D184	natural hybrid (*phalaenopsis* x *discolor*)	*P* x *C*	*superbiens*
D251	*lasianthera* x *bigibbum*	*C* x *P*	Mem. Edward Trevor
K580-3	*bigibbum* var. *compactum* 'K388-24' x *canaliculatum*	*P* x *E*	Mini Pearl

(Kamemoto, personal communication) with 0.01% colchicine to induce chromosome doubling (Sanguthai et al., 1973).

Untreated and colchicine-treated plants were compotted and grown in 2-inch pots, and then subsequently transferred to 6-inch pots. Ten plants of each ploidy level were grown in the greenhouse facility of the Mauka Manoa Campus for evaluation. Ploidy levels were verified by taking chromosome counts of the plants in 2-inch or 6-inch pots.

3.1.2 Characteristics Evaluated

Yield and inflorescence characteristics. Yield was measured once a month as the number of racemes produced during the evaluation period. Racemes with at least 2/3 of the flowers open were counted for the month. The number of flowers per spray and the number of buds dropped were counted.

Derived measurements were the average number of flowers per spray, percent buds dropped, percent sprays with bud drop, and percent distorted sprays. The percent buds dropped was calculated as the ratio of the number of buds that dropped to the sum of the dropped buds and the number of flowers left on the spray at the time of observation. The percent sprays with bud drop

is the ratio of the number of sprays with bud drop to the total number of sprays produced.

Raceme length was measured from the point of attachment on the pseudobulb to the tip of the spray. Scape length was measured from the base of the raceme to the first flower.

Racemes with 8 or more flowers were used for vase life evaluation and were cut when 2/3 to 3/4 of the flowers were open. At least 40 racemes from each selection and ploidy were evaluated from April 1985 to October 1986. Stems were held in tap water in 500 ml Erlenmayer flasks and were recut every other day. Vase life was expressed as the number of days until 50 percent of the flowers on the raceme wilted or dropped off.

Distorted sprays have been observed in the tetraploid forms of D. xLowana Nioka and D. xNeo-Hawaii. Thus, the percentage of distorted sprays, which is the sum of distorted sprays relative to the total number of sprays produced, was also recorded.

Flower size. Flower size was determined by taking the natural spread of the third flower from the base of the raceme. The natural spread consisted of the flower's length and width. Petal length and width,

dorsal sepal length and width, and pedicel length were also measured.

Pseudobulb height and number. Upon termination of yield evaluation, the height of the tallest pseudobulb and the number of pseudobulbs were taken.

Statistical analysis. Mean comparisons between ploidy levels for each selection were performed using Student's t-test.

3.2 Sporad Formation and Crossability of Diploid Species Hybrids and Their Corresponding Amphidiploids

3.2.1 Sporad Formation

Pollinia at post-meiotic metaphase were fixed and treated in a modified Carnoy's mixture of 1:1:2 chloroform: 95% ethyl alcohol: glacial acetic acid mixture for 20-30 min at room temperature. The pollinia were transferred to 45% acetic acid for 20-30 min, squashed and stained in 1% aceto-orcein (Kamemoto et al., 1964). When immediate squashing was not possible, the pollinia were stored in the fixative at 1-5C in the freezer.

500 sporads were observed and classified. The number of chromosomes in the microcytes were tallied as

an indication of the frequencies of meiotic abnormalities.

3.2.2 Crossability

Two diploid species, D. rumphianum and D. stratiotes, of the section Ceratobium were used as test parents because of their numerous flowers available for setting seed. Five pollinations were attempted in June 1988 for each selection within each ploidy level. Reciprocal crosses were carried out. The date of pollination and the date of harvest were recorded. Fruits were harvested about 3 months from pollination, which is usually adequate for fertilization and embryo development to take place (Niimoto and Sagawa, 1961; Sagawa and Valmayor, 1966).

The percentage of seed set and non-aborted embryos were used as measures of fertility. The percentage of non-aborted embryos of each pod were estimated by observing a random sample of 300 seeds under the microscope. Pod size was noted.

3.3 Evaluation of Progenies of Some Induced Amphidiploids and Their Corresponding Diploid Counterparts for the Development of Seed-Propagated Cultivars

The D. xJaquelyn Thomas-type amphidiploids Y166-1, 0580, Y972, and D168-12 have been previosly used to

produce seed-propagated commercial cutflower cultivars with relatively uniform progeny with desirable characters (Kamemoto and McConnell, 1984; Kamemoto, 1985). Since amphidiploid selections of K461-22, K522-1, K523-4 and K524-135 are the same as Y166-1, O580, Y972 and D168-12, respectively, their progenies were not evaluated.

Four newly induced amphidiploids with potential to produce seed-propagated cultivars, 'K333-22' (D. xJaquelyn Thomas), 'D251' (D. xMem. Edward Trevor), 'K432-2' (D. xAutumn Lace) and 'D184' (D. superbiens) were evaluated. K333-22 was selected from a cross between a blue D. gouldii 'D158' and D. phalaenopsis 'H2-4' (D. phalaenopsis 'Kosaki' X D. phalaenopsis var. alba). Unlike other D. xJaquelyn Thomas, K333-22 is short-statured and has racemes with short scapes and may therefore have potential to produce flowering potted plant cultivars. Six of its progenies were evaluated (Table 3.2). 'D251' is also short-statured with possible use in breeding flowering potted plants. However it produces droopy sprays. Five of its progenies were evaluated (Table 3.2). 'K432-2' is another short-statured floriferous selection with potential for breeding flowering potted plant cultivars. Five of its progenies were evaluated (Table 3.3).

When the natural hybrid D. superbiens (D184) is crossed with D. phalaenopsis, the hybrid is called D. xLouis Bleriot. 'Louis Bleriot' is a triploid commercial clone for production of individual flowers for leis. However, most of the 'Louis Bleriot' clones in cultivation are likely to be virus-infected (Kamemoto, personal communication). Hence, if D. xLouis Bleriot can be seed-propagated by utilizing amphidiploidy, virus-free seedlings may be produced. Seven crosses are shown in Table 3.4. The pollen parent for both crosses K1293 and K1298 is D. phalaenopsis 'D354-3'. However, the female parent of K1293 was D184 4N, while that of K1298 was D184 2N. Similarly, K1319 resulted from D184 4N and D. phalaenopsis var. compactum 'D356-1', while K1320 resulted from D184 2N with 'D356-1'.

3.3.1 Observations on Variability

General observations on seed viability, flower uniformity and plant vigor were noted. Photographs were taken to document the degree of variability of the offspring. Flower size, pedicel, scape, raceme length and pseudobulb height were measured in crosses with K333-22 and D251 to be used as potted plants. Flower size and pedicel length were measured for crosses with D. superbiens (Table 3.4).

Table 3.2. Crosses with K333-22 and D251.

Amphidiploid Parent	Cross Number	Parentage
K333-22, *D.* xJaquelyn Thomas	K1031	K333-22 x *D.*xJaquelyn Thomas 'O580 4N'
	K1103	K333-22 x *D.* *bigibbum* 'K388-24'
	K1121	K333-22 x K404-2
	K1122	K333-22 x *D.* xMae Teramoto 'K315-5'
	K1156	K333-22 x *D.* xMay Neal 4N
	K1190	K333-22 x *D.* *phalaenopsis* var. *compactum* 'D356-1'
D251, *D.* xMem. Edward Trevor	K997	D251-4N x *D.* *bigibbum* 'K297-2N'
	K1033	D251-4N x *D.* xMae Teramoto 'K315-17'
	K1133	D251-4N x *D.* phalaenopsis 'K577-16'
	K1136	D251-4N x K404-2

Table 3.3. Crosses involving diploid and amphidiploid D. xAutumn Lace, K432-2.

Cross Number	Parentage
K1254	K432-2-4N x D. bigibbum
K1255	K432-2-4N x D. tangerinum
K1256	K432-2-4N x D. stratiotes
K1257	K432-2-4N x D. antennatum
K1258	K432-2-2N x D. stratiotes

Table 3.4. Crosses with diploid and amphidiploid *D. superbiens*, D184 and *D. phalaenopsis* to produce *D.* xLouis Bleriot.

Cross Number	Parentage
K1287	D184-2N x *D. phalaenopsis*, D354-4
K1291	D184-2N x *D. phalaenopsis* 'Kosaki'
K1292	D184-2N x *D. phalaenopsis*, D390-6
K1293	D184-4N x *D. phalaenopsis*, D354-3
K1298	D184-2N x *D. phalaenopsis*, D354-3
K1319	D184-4N x *D. phalaenopsis* var. *compactum*, D356-1
K1320	D184-2N x *D. phalaenopsis* var. *compactum*, D356-1

3.4 Androgenesis in Crosses Involving D. xLouis Bleriot and D. xJaquelyn Thomas and D. xNeo-Hawaii Amphidiploids

3.4.1 Plant Materials

In 1985, 11 crosses were made involving three different clones of D. xLouis Bleriot and four different PPCC amphidiploids which have been used to produce University of Hawaii-released seed propagated cultivars: D. xJaquelyn Thomas 'Y166-1', D. xNeo Hawaii 'Y972 4N', D. xJaquelyn Thomas 'O580 4N', and D. xJaquelyn Thomas 'D168-12' (Table 3.5). Crosses were made in February 1985, sown in June 1985 and compotted in January 1986. From each cross, 64 individuals were grown in 2-inch pots for evaluation in the greenhouse at the Mauka Campus of the University of Hawaii at Manoa.

3.4.2 Chromosome Counts

Counts were made using the root tip squash method of Tanaka and Kamemoto (1984). Root tips were collected between 8 and 10 am, and pretreated with 0.002 M 8-hydroxy-quinoline at 10C for three to five hours. These were fixed with a modified Carnoy's solution of 1:1:2 chloroform, 95% ethanol and glacial acetic acid for 15 min. Root tips were hydrolyzed with 1N HCl for 3 min at 60C. The root tips were rinsed

Table 3.5. Crosses evaluated for the occurrence of androgenesis.

Female Parent	Amphidiploid Male Parent			
Louis Bleriot Clone	Y166-1	O580	Y972	D168-12
LBH	K1056	K1057	K1058	K1059
LBK	K1060	K1061	K1062	K1063
LBU		K1064	K1065	K1066

with water and stored in 45% acetic acid. They were squashed and stained in 1% aceto-orcein for observation.

3.4.3 Cymbidium Mosaic Virus (CyMV) Assay

Leaf samples from the androgenetic diploids were assayed for Cymbidium Mosaic Virus by the University of Hawaii Plant Disease Clinic using ELISA methods.

4. RESULTS AND DISCUSSION

4.1 Morphology and Yield of Diploid Dendrobium Species Hybrids and Their Corresponding Amphidiploids

4.1.1 Inflorescence and Yield Characteristics

The raceme length of the amphidiploids was shorter than that of the diploids in four pairs, longer in one and the same in six (Table 4.1). In only one pair was the scape length shorter (K227-27). Three of the pairs with a reduction in raceme length in the amphidiploid, plus two other pairs showed a reduction in the number of flowers per raceme.

Four of the 11 amphidiploids produced fewer racemes than the corresponding diploid, while seven showed no reduction. This is partially in accord with earlier observations that the polyploid forms are less floriferous (Wimber and Wimber, 1968; Kam and Kamemoto, 1980; Chaicharoen and Saejew, 1981; Leonhardt, 1986).

There was a significant difference in bud drop percentage for four pairs of selections. In two, bud drop was higher in the diploids; in the other two, it was higher in the amphidiploids. The percent of racemes with bud drop also varied. In two pairs the diploids had more racemes with bud drop, while in three pairs the amphidiploids had more. The biggest difference in percent racemes with bud drop was in D184 with 1.8% in the diploid and 20.4% in the amphidiploid.

Table 4.1. Raceme and yield characteristics of diploid and amphidiploid Dendrobium species hybrids.

Hybrid Selection	Ploidy Level	Observation Period	Raceme Length (cm)	Scape Length (cm)	Number of Flowers per Raceme	Number of Racemes Produced	Bud Drop (%)	% Racemes with Bud Drop
K227-27	2N	1/84 - 12/85	38.4 **	14.4 **	16.9 **	37.1 ns	1.4 ns	16.52 ns
	4N		33.4	12.2	14.5	31.1	1.4	14.72
K228-21	2N	1/84 - 12/85	34.5 *	13.6 ns	27.4 **	5.6 **	5.5 ns	41.80 ns
	4N		30.4	12.9	22.7	2.6	2.4	29.63
K432-2	2N	6/88 - 7/90	24.5 ns	14.4 ns	11.9 ns	19.2 ns	3.6 *	27.61 **
	4N		23.1	15.1	11.8	19.2	6.6	43.71
K333-22	2N	2/85 - 9/88	33.5 **	9.4 ns	18.3 **	27.3 ns	11.3 *	55.52 **
	4N		22.5	7.5	14.7	19.7	6.3	35.62
K461-22	2N	8/84 - 12/86	63.0 *	16.8 ns	18.8 ns	25.6 **	1.4 ns	14.61 **
	4N		61.4	17.2	18.6	12.4	1.9	24.59
K522-1	2N	8/84 - 12/86	43.2 **	16.3 ns	12.3 ns	23.5 ns	0.8 ns	8.35 ns
	4N		48.3	18.5	11.6	25.8	0.9	7.54

ns = not significant
* Mean difference significant at 5% level
** Mean difference significant at 1% level

Table 4.1. (Continued) Raceme and yield characteristics of diploid and amphidiploid *Dendrobium* species hybrids.

Hybrid Selection	Ploidy Level	Observation Period	Raceme Length (cm)	Scape Length (cm)	Number of Flowers per Raceme	Number of Racemes Produced	Bud Drop (%)	% Racemes with Bud Drop
K523-4	2N	8/84 - 12/86	60.1 ns	21.4 ns	17.1 ns	19.4 ns	1.4 ns	19.05 ns
	4N		53.7	20.4	15.9	15.2	1.4	17.06
K524-135	2N	8/84 - 12/86	58.4 ns	12.9 ns	23.0 **	23.7 ns	6.4 *	58.79 ns
	4N		53.7	12.9	19.7	17.7	13.2	66.07
D184	2N	6/88 - 7/90	39.2 ns	15.5 ns	15.1 *	4.4 ns	0.3 *	1.8 **
	4N		37.5	16.8	11.6	3.7	2.0	20.4
D251	2N	5/84 - 1/87	36.4 ns	15.4 ns	12.7 ns	21.3 **	5.7 ns	39.57 ns
	4N		32.7	14.4	12.0	13.5	6.0	40.30
K580-3	2N	2/86 - 3/88	27.8 ns	15.0 ns	12.2 ns	54.7 **	10.2 ns	54.22 *
	4N		30.0	16.1	11.4	41.1	9.3	45.25

ns = not significant
* Mean difference significant at 5% level
** Mean difference significant at 1% level

4.1.2 Vase Life and Distortion

Although and increase in ploidy has been reported to increase the substance and postharvest life of flowers (Mehlquist, 1949), no differences in vase life between diploids and amphidiplods were observed (Table 4.2). For Dendrobium, a vase life of 12-14 days or more is desired (Kamemoto, 1980). Only K333-22 2N and both 2N and 4N D251 had shorter vase lives.

Distortion of the racemes was observed in selections K227-27 (D. xLowana Nioka) and K523-4 (D. xNeo-Hawaii) (Table 4.3). Distorted racemes greatly increased in the amphidiploid forms of both selections. Kamemoto (1980) and later McConnell (1984) reported that tetraploid D. xNeo-Hawaii 'Y972' produced more distorted racemes than its corresponding diploid.

4.1.3 Flower Characteristics

Flower size either increased or remained the same as the ploidy level increased (Table 4.4). In most selections, increased size was due to broader flowers, as well as longer and/or broader petals and sepals. Similar observations have been reported in Cymbidium (Wimber and Wimber, 1967; Leonhardt, 1967), Aranda (Kam and Kamemoto, 1980) and Dendrobium (McConnell, 1980). Petal measurements for the Ceratobium-Eleutheroglossum

Table 4.2. Vase life of diploid and amphidiploid *Dendrobium* selections.

Hybrid Selection	Ploidy Level	Half-life (days)
K227-27	2N	21.9 ns
	4N	24.0
K228-21	2N	14.9 ns
	4N	14.6
K333-22	2N	10.5 ns
	4N	12.8
K461-22	2N	14.5 ns
	4N	15.8
K522-1	2N	15.3 ns
	4N	14.9
K523-4	2N	15.1 ns
	4N	14.2
K524-135	2N	12.4 ns
	4N	14.6
D251	2N	9.8 ns
	4N	11.2

ns = not significant
* Mean difference significant at 5% level
** Mean difference significant at 1% level

Table 4.3. Raceme distortion in K227-27 and K523-4.

Hybrid Selection	Ploidy Level	Percent Distorted Racemes
K227-27	2N	0.84 **
	4N	15.93
K523-4	2N	4.88 **
	4N	31.82

** Mean difference significant at 1% level

Table 4.4. Flower, sepal, petal, and pedicel measurements of diploid and amphidiploid *Dendrobium* selections. All measurements in cm.

Hybrid Selection	Ploidy Level	Flower		Dorsal Sepal		Petal		Pedicel
		Length	Width	Length	Width	Length	Width	Length
K227-27	2N	3.5 ns	3.9 **	2.3 ns	0.4 **	...	...	2.4 ns
	4N	3.6	4.2	2.3	0.6	...	...	2.5
K228-21	2N	3.8 ns	3.3 **	2.4 **	0.5 **	...	...	2.8 **
	4N	4.1	4.1	2.7	0.7	...	...	3.2
K432-2	2N	3.9 ns	4.0 ns	2.4 ns	0.5 **	...	...	2.8 **
	4N	4.0	4.1	2.4	0.8	...	...	3.0
K333-22	2N	4.3 ns	5.0 ns	2.9 **	1.0 **	3.4 ns	1.5 **	3.8 *
	4N	4.4	5.0	3.5	1.6	2.9	2.1	3.5
K461-22	2N	4.2 *	4.8 **	3.2 **	0.9 **	3.6 *	1.2 **	4.4 **
	4N	4.8	5.5	3.4	1.0	3.8	1.4	4.8
K522-1	2N	4.3 **	5.0 ns	3.4 ns	1.0 ns	3.8 ns	1.5 ns	4.7 **
	4N	5.0	5.1	3.6	1.1	3.9	1.5	5.3

ns = not significant
* Mean difference significant at 5% level
** Mean difference significant at 1% level

Table 4.4. (Continued) Flower, sepal, petal, and pedicel measurements of diploid and amphidiploid Dendrobium selections. All measurements in cm.

Hybrid Selection	Ploidy Level	Flower		Dorsal Sepal		Petal		Pedicel
		Length	Width	Length	Width	Length	Width	Length
K523-4	2N	5.3 ns	5.2 ns	3.4 ns	1.0 **	4.0 ns	1.5 *	4.4 **
	4N	5.4	5.8	3.6	1.3	4.1	1.9	4.8
K524-135	2N	4.5 ns	4.6 **	3.1 ns	0.9 **	3.5 ns	1.2 **	4.6 ns
	4N	4.7	5.0	3.1	1.1	3.6	1.4	4.6
D184	2N	3.9 **	4.7 **	2.7 **	1.1 ns	3.3 *	1.8 **	3.8 ns
	4N	4.4	5.2	3.0	1.2	3.5	2.2	3.7
D251	2N	5.5 ns	5.1 **	3.4 **	0.9 **	4.0 ns	1.1 **	3.7 ns
	4N	5.9	5.8	3.7	1.2	4.2	1.5	4.0
K580-3	2N	2.7 **	3.6 **	2.0 **	0.6 **	2.5 **	0.9 **	2.8 ns
	4N	3.6	4.1	2.5	0.8	2.9	1.3	2.7

ns = not significant
* Mean difference significant at 5% level
** Mean difference significant at 1% level

selections (K227-27, K228-21 and K432-2) were not taken because their petals were twisted and reflexed.

Pedicel length usually increased with increase in ploidy level. In selections K228-21, K432-2, K461-22, K522-1 and K523-4, the pedicel of the amphidiploids was longer, while in K333-22, the diploid was longer. Short pedicels contribute to the compactness of the raceme. When the increase in pedicel length is accompanied by a proportional increase in flower size, a slight increase in pedicel length may not be undesirable.

4.1.4 Pseudobulb Characteristics

Shorter pseudobulbs were produced by two amphidiploids while taller pseudobulbs were produced by one amphidiploid compared to their corresponding diploids (Table 4.5).

The number of pseudobulbs appeared to decrease with an increase in chromosome number. However, statistical significance was obtained for only K227-27.

Table 4.5. Pseudobulb height and number of diploid and amphidiploid Dendrobium selections.

Hybrid Selection	Ploidy Level	Pseudobulb Height (cm)	Pseudobulb Number
K227-27	2N	30.3 **	16.4 **
	4N	38.0	10.0
K228-21	2N	41.3 ns	8.2 ns
	4N	42.5	6.0
K432-2	2N	32.5 ns	10.9 ns
	4N	38.7	10.8
K333-22	2N	58.1 *	11.3 ns
	4N	51.7	10.9
K461-22	2N	150.4 **	7.4 ns
	4N	115.0	6.1
K522-1	2N	64.1 ns	16.2 ns
	4N	75.3	14.1
K523-4	2N	113.4 ns	7.3 ns
	4N	104.3	6.8
K524-135	2N	79.4 ns	11.3 ns
	4N	82.4	8.9
D184	2N	128.0 ns	7.3 ns
	4N	106.8	7.5
D251	2N	33.7 ns	12.3 ns
	4N	38.7	8.6
K580-3	2N	25.8 ns	14.4 ns
	4N	27.0	13.0

ns = not significant
* Mean difference significant at 5% level
** Mean difference significant at 1% level

4.2 Sporad Formation, Crossability and Breeding Behavior of Diploid Dendrobium Hybrids and Their Corresponding Amphidiploids

4.2.1 Sporad Formation

Sporad formation varied among intersectional hybrids and cultivars within each group (Table 4.6). Among the Ceratobium-Eleutheroglossum crosses, K227-27 and K432-2 behaved similarly. Diploids in both selections produced predominantly tetrads and some tetrads with microcytes. The amphidiploid counterparts produced fewer tetrads, and more tetrads with microcytes than their corresponding diploids. The high frequency of tetrads formed at the diploid level indicates relatively good pairing at meiosis, and close homology of chromosomes of the two parental species. The increase in microcyte formation in the amphidiploids suggests meiotic irregularities related to multivalent associations of homologous or homoeologous chromosomes.

Sporad formation in diploid K228-21 was highly irregular with the formation of only 27.2% tetrads and 64.2% tetrads with microcytes. The corresponding amphidiploid exhibited a high percentage of tetrads (96%). Thus, doubling of chromosomes resulted in a more uniform meiotic behavior from the highly irregular behavior in the diploid hybrid.

Table 4.6. Microspore formation in diploid Dendrobium species hybrids and their corresponding amphidiploids. (mc=microcytes; C=Ceratobium, E=Eleutheroglossum, P=Phalaenanthe; numbers in parenthesis are percentages).

Inter-sectional Hybrid	Genome	Tetrad	Tetrad + mc	Dyad	Dyad + mc	Others	Total
K227-28	CE	186 (84.9)	29 (13.2)	2 (0.4)	1 (0.2)	1 (0.2)	219 (100.0)
	CCEE	278 (55.6)	221 (44.2)	1 (0.2)			500 (100.0)
K228-21	CE	83 (27.2)	196 (64.2)	15 (4.9)	4 (1.3)	7 (2.3)	305 (100.0)
	CCEE	192 (96.0)	5 (2.5)	3 (1.5)			200 (100.0)
K432-2	CE	467 (93.4)	27 (5.4)	2 (0.4)	4 (0.8)		500 (100.0)
	CCEE	442 (88.4)	57 (11.4)		1 (0.2)		500 (100.0)
K333-22	PC	171 (85.5)	10 (5.0)	17 (8.5)	2 (1.0)		200 (100.0)
	PPCC	447 (89.4)	53 (10.6)				500 (100.0)
K461-22	PC	256 (51.2)	20 (4.0)	207 (41.4)	17 (3.4)		500 (100.0)
	PPCC	496 (99.2)	4 (0.8)				500 (100.0)
K522-1	PC	23 (4.6)		433 (86.6)	44 (8.8)	2 (0.4)	500 (100.0)
	PPCC	458 (91.6)	24 (4.8)	15 (3.0)	1 (0.2)	2 (0.4)	500 (100.0)

Table 4.6. (Continued) Microspore formation in diploid Dendrobium species hybrids and their corresponding amphidiploids. (mc=microcytes; C=Ceratobium, E=Eleutheroglossum, P=Phalaenanthe; numbers in parenthesis are percentages).

Inter-sectional Hybrid	Genome	Tetrad	Tetrad + mc	Dyad	Dyad + mc	Others	Total
K523-4	PC	265 (53.0)	25 (5.0)	173 (34.6)	34 (6.8)	3 (0.6)	500 (100.0)
	PPCC	469 (93.8)	29 (5.8)	1 (0.2)		1 (0.2)	500 (100.0)
K524-135	PC	93 (18.6)	6 (1.2)	372 (74.4)	23 (4.6)	6 (1.2)	500 (100.0)
	PPCC	486 (97.2)	13 (2.6)	1 (0.2)			500 (100.0)
D184	PC	452 (90.4)	36 (7.2)	11 (2.2)	1 (0.2)		500 (100.0)
	PPCC	441 (88.2)	59 (11.8)				500 (100.0)
D251	PC	430 (86.0)	22 (4.4)	32 (6.4)	5 (1.0)	11 (2.2)	500 (100.0)
	PPCC	490 (98.0)	1 (0.2)	7 (1.4)	1 (0.2)	1 (0.2)	500 (100.0)
K580-3	PE	453 (90.6)	27 (5.4)	19 (3.8)	1 (0.2)		500 (100.0)
	PPEE	361 (72.2)	136 (27.2)	3 (0.6)			500 (100.0)

Considerable variations in sporad formation were encountered among the Phalaenanthe-Ceratobium diploid hybrids. The percentage of tetrads produced ranged from the exceptionally low 4.6 for K522-1 to as high as 90.4 for the natural hybrid, D. superbiens (D184). A low percentage of tetrads was usually accompanied by a high percentage of dyads. K522-1 produced 4.6% tetrads and 86.6% dyads, and K524-135 produced 18.6% tetrads and 74.4% dyads. Dyad formation often results from irregular pairing and restitution of nucleus after the first meiotic division (Storey, 1956; Kosaki and Kamemoto, 1961).

It is interesting to note that, among the Phalaenanthe-Ceratobium hybrids, the natural hybrid D184 (D. superbiens), showed the highest percentage of tetrads (90.4%) as a diploid. Its amphidiploid produced 88.2% tetrads, very similar to the diploid. In the other Phalaenanthe-Ceratobium hybrids, doubling the chromosomes resulted in a high frequency of tetrad formation from 89.4% for K333-22 to 99.2% for K461-22. These high percentages are the results of preferential pairing of parental chromosomes and are associated with high fertilities that have allowed the successful production of seed-propagated amphidiploid cultivars (Kamemoto and McConnell, 1984; Kamemoto, 1985).

The diploid Phalaenanthe-Eleutheroglossum hybrid K580-3 formed mostly tetrads. Although predominantly tetrads were observed in the tetraploid counterpart, the percentage of tetrad formation was lower than that of the diploid. Furthermore, the frequency of tetrads with microcytes increased from 5.4% in the diploid to 27.2% in the amphidiploid.

The occurrence of microcytes in the amphidiploid counterpart may be due to intergenomal and multivalent pairing. McConnell (1984) reported that loose associations and univalents in the tetraploid D. xJaquelyn Thomas 'O580' resulted in irregular sporad formation. Microcytes were observed in individuals with high frequencies of univalents at Metaphase I.

4.2.2 Crossability of Diploid Dendrobium Hybrids and Their Corresponding Amphidiploids with D. rumphianum and D. stratiotes

Fruit set was low when Ceratobium-Eleutheroglossum selections were test crossed (Table 4.7). All selections failed as paternal parents except two; K228-21 4N crossed to D. stratiotes, and K432-2 4N, also crossed to D. stratiotes.

When D. rumphianum was used as a test parent, fruit set occurred in only four cross combinations and resulted in a low percentage of viable embryos, except

Table 4.7. Crossability of diploid and amphidiploid *Ceratobium-Eleutheroglossum* hybrids with *D. rumphianum* and *D. stratiotes*.

Cross	Number of Fruits Formed	Fruit Set (%)	Embryo Viability (%)
K227-27 2N X *D. rumphianum*	3	60	0
D. rumphianum X K227-27 2N	0	0	...
K227-27 4N X *D. rumphianum*	3	60	91
D. rumphianum X K227-27 4N	0	0	...
K228-21 2N X *D. rumphianum*	2	40	1
D. rumphianum X K228-21 2N	0	0	...
K228-21 4N X *D. rumphianum*	0	0	...
D. rumphianum X K228-21 4N	0	0	...
K432-2 2N X *D. rumphianum*	1	20	18
D. rumphianum X K432-2 2N	0	0	...
K432-2 4N X *D. rumphianum*	0	0	...
D. rumphianum X K432-2 4N	0	0	...
K227-27 2N X *D. stratiotes*	3	60	6
D. stratiotes X K227-27 2N	0	0	...
K227-27 4N X *D. stratiotes*	1	20	92
D. stratiotes X K227-27 4N	0	0	...
K228-21 2N X *D. stratiotes*	3	60	5
D. stratiotes X K228-21 2N	0	0	...
K228-21 4N X *D. stratiotes*	4	80	62
D. stratiotes x K228-21 4N	3	60	31
K432-2 2N X *D. stratiotes*	3	60	33
D. stratiotes X K432-2 2N	0	0	...
K432-2 4N X *D. stratiotes*	0	0	...
D. stratiotes X K432-2 4N	1	20	60

for the cross with K227-27 4N. Although the crosses with K227-27 2N and K227-27 4N both resulted in fruit set, K227-27 4N produced viable embryos.

Seven cross combinations resulted in fruit set when D. stratiotes was used. Crosses with all diploids resulted in 60% fruit set and 5% (K228-21 2N) to 33% (K432-2 2N) viable embryos. More viable embryos (62% for K432-2 4N to 92% for K227-27 4N) were produced by the amphidiploid crosses. The comparisons for K227-27 and K228-21 support the hypothesis that doubling results in increased fertility.

Although most Ceratobium-Eleutheroglossum diploid hybrids exhibit partial female fertility, they are highly male sterile. However, the amphidiploids usually exhibited a greater percentage of viable embryos, especially in crosses with D. stratiotes. The high degree of male sterility in most of the Ceratobium-Eleutheroglossum crosses observed may be due to factors other than irregularities in microspore formation. The higher tetrad formation observed in the amphidiploids of all three selections (Table 4.6) would have been expected to produce increased fertility even when used as the pollen parent.

Fruit set was higher in test crosses with the Phalaenanthe-Ceratobium selections (Tables 4.8 and

Table 4.8. Crossability of diploid and amphidiploid *Phalaenanthe-Ceratobium* hybrids with *D. rumphianum*.

Cross	Number of Fruits Formed	Fruit Set (%)	Embryo Viability (%)
K333-22 2N X *D. rumphianum*	3	60	13
D. rumphianum X K333-22 2N	0	0	...
K333-22 4N X *D. rumphianum*	4	80	91
D. rumphianum X K333-22 4N	0	0	...
K461-22 2N X *D. rumphianum*	0	0	...
D. rumphianaum X K461-22 2N	0	0	...
K461-22 4N X *D. rumphianum*	0	0	...
D. rumphianaum X K461-22 4N	0	0	...
K522-1 2N X *D. rumphianum*	5	100	0
D. rumphianum X K522-1 2N	0	0	...
K522-1 4N X *D. rumphianum*	4	80	8
D. rumphianum X K522-1 4N	0	0	...
K523-4 2N X *D. rumphianum*	0	0	...
D. rumphianum X K523-4 2N	0	0	...
K523-4 4N X *D. rumphianum*	1	20	32
D. rumphianum X K523-4 4N	0	0	...
K524-135 2N X *D. rumphianum*	4	80	6
D. rumphianum X K524-135 2N	0	0	...
K524-135 4N X *D. rumphianum*	5	100	88
D. rumphianum X K524-135 4N	0	0	...
D251 2N X *D. rumphianum*	0	0	...
D. rumphianum X D251 2N	0	0	...
D251 4N X *D. rumphianum*	3	60	15
D. rumphianum X D251 4N	0	0	...

4.9). All selections failed as pollen parents, with the exception of K522-1 4N and K524-135 when crossed to D. stratiotes. Twenty-one of the 24 crosses using the selections as the female produced fruit set. Although the differences in fruit set between the diploid and amphidiploid pairs was often small, the percentage of viable embryos were considerably higher in many of the amphidiploids. An example is in the crosses of K333-22 with D. rumphianum (Table 4.8), where the diploid produced 13% viable embryos, while the amphidiploid produced 91%.

The only diploid Phalaenanthe-Ceratobium selection that had good fertility was K523-4 2N, but only when crossed to D. stratiotes. The dyads produced by this plant must have been functional, similar to the behavior of D. phalaenopsis 'Lyon's Light No. 1' (Kamemoto and Tara, 1968).

When the Phalaenanthe-Eleutheroglossum hybrid (K580-3) was crossed with either D. rumphianum or D. stratiotes (Table 4.10), no fruits were formed when K580-3 was used as the pollen parent. Fruit set was observed in all crosses when K580-3 either 2N or 4N was used as a seed parent. However, more viable embryos were produced by K580-3 4N.

In summary, most test crosses of amphidiploid Ceratobium-Eleutheroglossum, Phalaenanthe-Ceratobium

Table 4.9. Crossability of diploid and amphidiploid *Phalaenanthe-Ceratobium* hybrids with *D. stratiotes*.

Cross	Number of Fruits Formed	Fruit Set (%)	Embryo Viability (%)
K333-22 2N X *D. stratiotes*	2	40	0
D. stratiotes X K333-22 2N	0	0	...
K333-22 4N X *D. stratiotes*	4	80	67
D. stratiotes X K333-22 4N	0	0	...
K461-22 2N X *D. stratiotes*	5	100	9
D. stratiotes X K461-22 2N	0	0	...
K461-22 4N X *D. stratiotes*	5	100	97
D. stratiotes X K461-22 4N	0	0	...
K522-1 2N X *D. stratiotes*	5	100	0
D. stratiotes X K522-1 2N	0	0	...
K522-1 4N X *D. stratiotes*	5	100	47
D. stratiotes X K522-1 4N	1	20	90
K523-4 2N X *D. stratiotes*	5	100	85
D. stratiotes X K523-4 2N	0	0	...
K523-4 4N X *D. stratiotes*	5	100	97
D. stratiotes X K523-4 4N	0	0	...
K524-135 2N X *D. stratiotes*	5	100	0
D. stratiotes X K524-135 2N	0	0	...
K524-135 4N X *D. stratiotes*	0	0	...
D. stratiotes X K524-135 4N	2	40	98
D184 2N X *D. stratiotes*	5	100	29
D. stratiotes X D184 2N	0	0	...
D184 4N X *D. stratiotes*	1	20	80
D. stratiotes X D184 4N	...	...	...
D251 2N X *D. stratiotes*	3	60	6
D. stratiotes X D251 2N	0	0	...
D251 4N X *D. stratiotes*	5	100	27
D. stratiotes X D251 4N	0	0	...

Table 4.10. Crossability of K580-3 (*Phalaenanthe-Eleutheroglossum*) with *D. rumphianum* and *D. stratiotes*.

Cross	Number of Fruits Formed	Fruit Set (%)	Embryo Viability (%)
K580-3 2N X *D. rumphianum*	2	40	53
D. rumphianum X K580-3 2N	0	0	...
K580-3 4N X *D. rumphianum*	2	40	81
D. rumphianum X K580-3 4N	0	0	...
K580-3 2N X *D. stratiotes*	5	100	38
D. stratiotes X K580-3 2N	0	0	...
K580-3 4N X *D. stratiotes*	3	60	88
D. stratiotes X K580-3 4N	0	0	...

and Phalaenanthe-Eleutheroglossum selections with D. rumphianum and D. stratiotes showed more fruit set and/or embryo viability than the crosses with their diploid counterparts. The viable embryos observed in crosses with the diploid selections may have been formed by functional dyads resulting from the restitution of nuclei following the first division (Storey, 1956). The increased female fertility of the amphidiploids is what is expected following chromosome doubling of a sterile inter-sectional hybrid. However, the very low fruit set for both the diploid and amphi-diploid selections as pollen parents suggests there may be some male sterility due to factors other than meiotic abnormalities. Other factors which may have influenced fruit set include environmental effects, particularly high temperatures, and the pollen source, as seen in the differences between the crosses with D. rumphianum vs. D. stratiotes.

4.2.3 Breeding Behavior of Selected Amphidiploids

4.2.3.1 Breeding Behavior of K333-22, D. xJaquelyn Thomas

Variability in K333-22 crosses was reflected during the early stages of growth (Table 4.11). Seed pods of the crosses ranged from small to large. Embryo viability ranged from 15% (K1121) to 97% (K1103)and

Table 4.11. Pod size, embryo viability and uniformity of seedlings in crosses with amphidiploid K333-22 as the female parent.

Cross	Male Parent	Pod Size	Embryo Viability (%)	Germination	Remarks
K1031	D. xJaquelyn Thomas, 0580 4N	S	65	Poor	Seedlings in compot uniform and vigorous. Seedlings in 2-in pot vigorous.
K1103	D. bigibbum, K388-24	M	97	Good	Variable growth of seedlings.
K1121	K404-2	L	15	Good	Seedlings in compot uniform
K1122	D. xMae Teramoto, K315-5	L	25	Good	Seedlings in compot vigorous but variable.
K1156	D. xMay Neal 4N	M	57	Fair	Seedlings in compot vigorous, uniform. Seedlings in 2-in. pots vigorous.
K1190	D. phalaenopsis var. compactum D356-1	M	64	Good	Seedlings in compot vigorous and uniform; in 2-in pots uniform.

germination was highly variable. Growth was uniform and vigorous in the compot stage in crosses K1031, K1121, K1156 and K1190, but in K1103 and K1122 growth was variable.

Measurements of the inflorescence and flower were highly variable (Table 4.12). The coefficient of variation for scape length ranged from 15.67% for K1122 to 27.71% for K1156, while that of raceme length ranged from 20.47% for K1031 to 37.91% for K1156. Flower size was less variable. The flower size was least variable for K1121 (c.v. for length=5.67%, width=4.89%). The most variable was K1156 (c.v. for length=16.17%, width=14.00%).

Progenies of K333-22 were relatively short statured at first flower (Table 4.13). Average height ranged from 18.4 cm for K1190 to 39.8 cm for K1156. The final height of the crosses also remained short (24.6 cm in K1190 to 53.5 cm for K1103).

The flower color in K1031 ranged from two-tone lavender to purple, which is very variable. K1031 is a cross with D. xJaquelyn Thomas 'O580 4N' which in two previous crosses, has produced offspring uniform enough to be released as seed-propagated cultivars (Bobisud and Kamemoto, 1982; McConnell, 1984). Thus, the variability observed here must have come from K333-22.

Table 4.12. Variability in inflorescence and flower measurements of progeny of amphidiploid K333-22. All measurements in cm.

Cross Number	Variable	Minimum	Maximum	Mean	Standard Deviation	CV (%)
K1031	Scape	9.0	18.0	13.8	2.9	21.32
	Raceme	18.5	43.0	30.9	6.3	20.47
	Flower Length	4.3	5.7	5.0	0.4	7.85
	Flower Width	4.8	6.3	5.6	0.5	8.87
	Pedicel	3.3	5.4	4.7	0.6	11.96
K1103	Scape	4.5	19.0	14.5	3.0	21.57
	Raceme	28.0	59.0	42.0	8.7	20.84
	Flower Length	4.2	6.0	4.8	0.5	10.84
	Flower Width	4.5	6.0	5.4	0.4	6.87
	Pedicel	3.0	4.0	3.7	0.4	9.00
K1121	Scape	7.0	17.0	12.9	2.0	15.24
	Raceme	15.0	50.0	33.0	8.9	26.89
	Flower Length	5.0	6.0	5.9	0.3	5.67
	Flower Width	6.0	7.0	6.8	0.3	4.89
	Pedicel	3.0	5.0	4.0	0.4	9.81
K1122	Scape	9.0	16.0	12.4	1.9	15.67
	Raceme	15.0	46.0	32.2	8.5	26.37
	Flower Length	5.0	6.0	5.0	0.4	5.94
	Flower Width	3.0	7.0	6.6	0.8	12.84
	Pedicel	3.0	4.0	3.8	0.4	10.96
K1156	Scape	5.0	20.0	13.0	3.0	27.71
	Raceme	7.0	45.0	25.4	9.5	37.91
	Flower Length	4.0	7.0	5.3	0.8	16.17
	Flower Width	4.0	7.0	5.0	0.8	14.00
	Pedicel	3.0	5.0	4.3	0.5	12.41
K1190	Scape	4.0	12.0	9.1	2.3	25.09
	Raceme	9.0	36.0	21.6	8.0	36.86
	Flower Length	4.0	5.0	4.7	0.3	6.61
	Flower Width	5.0	6.0	5.7	0.4	6.72
	Pedicel	2.0	4.0	3.0	0.4	11.05

Table 4.13. Variability in pseudobulb height of progeny of K333-22 4N at first flower and upon termination. All measurements in cm.

Cross	Height at First Flower and Termination	Minimum	Maximum	Mean	Standard Deviation	CV (%)
K1031	First flower	15.0	52.0	28.0	6.0	23.95
	Final height	22.0	78.0	47.5	13.4	28.27
K1103	First flower	12.0	42.0	26.0	8.0	30.99
	Final height	34.0	73.0	53.5	11.8	21.98
K1121	First flower	15.0	36.8	24.9	6.1	24.00
	Final height	26.0	49.5	40.2	5.4	13.37
K1122	First flower	10.0	42.0	26.2	7.8	29.93
	Final height	25.0	45.0	33.0	5.2	15.57
K1156	First flower	19.0	87.0	39.8	18.6	46.78
	Final height	30.0	87.0	47.8	15.8	32.96
K1190	First flower	8.0	27.0	18.4	6.1	33.0
	Final height	13.0	28.0	21.6	4.8	22.06

The variable offspring produced by K333-22 may be a result of intergenomal pairing, which may not have occurred in the other D. xJaquelyn Thomas amphidiploids producing uniform progeny. The existence of meiotic irregularity in amphidiploid K333-22 is also suggested by the production of 10.6% tetrads with microcytes (Table 4.6).

The most variable of the K333-22 crosses both in measurements and color (Figure 1) was K1156. Its male parent is a tetraploid D. xMay Neal, a complex species hybrid. It appears that D. xMay Neal also contributes variable products of meiosis to its progeny.

Of the six crosses observed, K1121 had the least variation in its measurements as well as in flower color (Figure 2). The pollen parent of K1121 is K404-2, a Phalaenanthe tetraploid (PPPP) with dark purple flowers. Therefore the two sets of chromosomes it contributes to its offspring resulted in the relatively uniform progeny. This cross shows considerable potential as a good seed-propagated potted plant cultivar because of its attractive large reddish purple flowers, good keeping quality of flowers on the plants and relatively short stature (Figure 2).

Hence, in five out of six crosses, amphidiploid K333-22 was not a desirable parent for the production

Figure 1. Flower color variation in K1156, D. xJaquelyn Thomas 'K333-22 4N' x D. xMay Neal 4N.

Figure 2. Flower color in K1121, D. xJaquelyn Thomas 'K333-22 4N' x K404-2.

of seed-propagated potted plant cultivars because of the high variability observed in its offspring.

4.2.3.2 Breeding Behavior of D251, D. xMem. Edward Trevor

Offspring of D251 exhibited variable pod size (Table 4.14), like the K333-22 offspring. Embryo viability ranged from 19 to 80% and germination was good in three of the four crosses. Early observations during compot indicated fairly uniform vegetative growth.

Inflorscence and flower measurements exhibited great variability (Table 4.15). The quality of the racemes was poor. The flowers were large with variable shape and color and were borne on long droopy racemes, similar to the female parent D251.

Although the progenies were shorter-statured than those of K333-22, plant heights was almost as variable (Table 4.16).

Because of high variability and poor quality sprays, D251 was not suitable for developing new seed-propagated potted plant cultivars.

Table 4.14. Pod size, embryo viability and uniformity of germination in crosses with amphidiploid D251 as the female parent.

Cross	Male Parent	Pod Size	Embryo Viability (%)	Germination	Remarks
K997	D. bigibbum 2N, D297	M	19	Good	Seedlings in compot vigorous; in 2-in. pot vigorous, short growth.
K1033	D. xMae Teramoto, K315-7	S	80	Good	Seedlings in compot vigorous; in 2-in. pot, uniform.
K1133	D. phalaenopsis 4N, K577-16	L	34	Good	Seedlings in compot uniform, vigorous.
K1136	K404-2 4N	S	60	Fair	Seedlings in compot uniform, vigorous.

Table 4.15. Variability in inflorescence and flower measurements of progeny of D251 4N. All measurements in cm.

Cross Number	Variable	Minimum	Maximum	Mean	Standard Deviation	CV (%)
K997	Scape	12.0	20.0	16.8	2.3	13.62
	Raceme	18.0	58.0	33.8	10.1	30.02
	Flower Length	4.0	6.0	5.1	0.5	10.26
	Flower Width	4.0	7.0	5.0	0.8	12.91
	Pedicel	2.0	4.0	3.6	0.8	20.92
K1033	Scape	14.0	33.0	21.1	3.0	18.46
	Raceme	19.0	52.0	38.3	7.7	19.99
	Flower Length	5.0	7.0	6.3	0.5	8.31
	Flower Width	5.0	8.0	7.1	0.8	11.33
	Pedicel	3.0	4.0	3.9	0.4	10.26
K1133	Scape	11.0	22.0	17.0	3.1	18.42
	Raceme	24.0	68.0	41.3	11.4	27.49
	Flower Length	5.0	7.0	6.3	0.5	7.42
	Flower Width	6.0	8.0	7.5	0.6	8.45
	Pedicel	3.0	5.0	4.1	0.5	11.31
K1136	Scape	12.0	22.0	15.9	2.5	15.64
	Raceme	24.0	49.0	33.0	6.2	18.39
	Flower Length	5.0	7.0	6.3	0.6	8.94
	Flower Width	6.0	8.0	7.0	0.7	8.86
	Pedicel	13.0	4.0	3.0	0.65	14.59

Table 4.16. Variability in pseudobulb height of crosses with D251 at first flower and upon termination. All measurements in cm.

Cross	Height at First Flower and Termination	Minimum	Maximum	Mean	Standard Deviation	CV (%)
K997	First flower	8.0	33.0	15.1	6.0	40.08
	Final height	9.0	43.0	22.8	9.0	42.00
K1033	First flower	11.0	39.0	22.6	5.9	26.28
	Final height	23.0	57.0	34.8	7.6	21.99
K1133	First flower	14.0	28.0	20.7	3.6	17.63
	Final height	24.0	55.0	34.1	7.8	22.74
K1136	First flower	14.0	28.0	22.0	3.5	16.0
	Final height	20.0	36.0	28.0	4.7	16.0

4.2.3.3 Breeding Behavior of K432-2, D. xAutumn Lace

The crosses with K432-2 showed fairly high embryo viability (60-82%) when the amphidiploid was the parent, but only 27% viability when the diploid was the parent (Table 4.17).

The flower color was uniform in the crosses of K432-2 4N with D. bigibbum, D. tangerinum, and D. antennatum. However, some variation in the crosses with D. stratiotes was observed (Figure 3). It can be seen that there was greater variability in K1258, the progeny of diploid K432-2 than in K1256, the progeny of the amphidiploid K432-2. Some intergenomal pairing or multivalent formation in the amphidiploid is suggested by this, which may mean that the parental genomes of K432-2, D. strebloceras (Ceratobium) and D. canaliculatum (Eleutheroglossum) are fairly homologous, and that K432-2 may be a segmental amphidiploid.

K1257 and K1258 showed vigorous seedling growth. Undesirable plant characteristics such as extensive bud drop, susceptibility to phytophthora and weak stem bases were observed in K1254, K1255 and K1256.

In summary, K432-2, D. xAutumn Lace was not a desirable parent for the production of seed-propagated cultivars because of the variability of the offspring, presumably due to intergenomal pairing.

Table 4.17. Embryo viability and flower uniformity of seedlings in crosses with K432-2, D. xAutumn Lace.

Cross Number	Parentage	Embryo Viability (%)	Uniformity of Flowers	Remarks
K1254	K432-2-4N X D. bigibbum	66	Uniform color	Floriferous; Taller plants in greenhouse. Extensive bud drop.
K1255	K432-2-4N X D. tangerinum	82	Uniform color	Tall, crooked pseudobulbs in greenhouse; shorter erect pseudobulbs in saranhouse. Several plants died from phytophthora in saranhouse.
K1256	K432-2-4N X D. stratiotes	80	Slightly variable	Floriferous; Plants with weak stem base.
K1257	K432-2-4N X D. antennatum	60	Uniform color	Uniform, vigorous growth of seedlings.
K1258	K432-2-2N X D. stratiotes	27	Slightly variable	Floriferous; Uniform vigorous growth of seedlings.

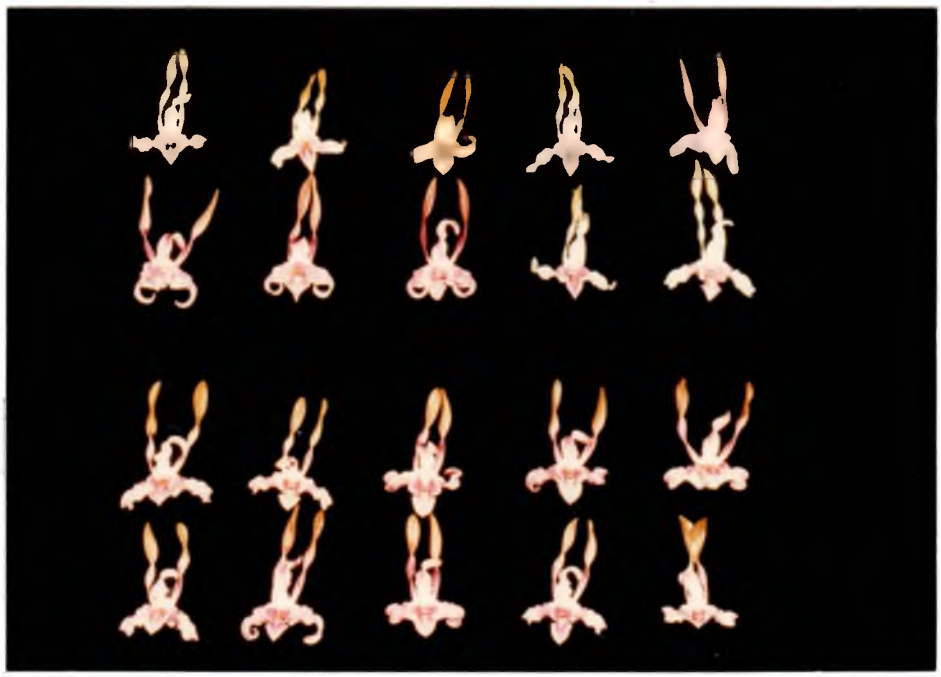

Figure 3. Flower color variation in K432-2, D. xAutumn Lace x D. stratiotes crosses. Upper two rows: K1258 progeny from K432-2 2N. Bottom two rows: K1256 progeny from K432-2 4N.

4.2.3.4 Breeding Behavior of D184, Dendrobium superbiens

Seed pods from crosses with D184 2N produced lower percecntages of viable seed, than did crosses with D184 4N (Table 4.18). K1298 (D184 2N x D354-3) had 50% viability while K1293 (D184 4N x D354-3) had 76%. Likewise, K1320 (D184 2N x D356-1) had 76%, while K1319 had 96%.

Progenies of D184 2N generally had highly variable flower measurements (Table 4.19). K1293, the only offspring of D184 4N measured, had the most uniform flower measurements. Although it did not produce the largest flowers, it was the most uniform for all the flower characteristics evaluated, except it was second most uniform for dorsal sepal width. Because of its uniformity, this cross has a potential to be used as a seed-propagated D. xLouis Bleriot.

Flower color was highly variable in the crosses with diploid D184, varying from shades of lavender and purple in K1291 (Figure 4), K1298, and K1320 to some bronze hues in K1287 and K1292. However, flower color was uniform in the crosses with amphidiploid D184 (K1293, as shown in Figure 4, and K1319). Since hybrids with D184 4N are more uniform than those of D184 2N, preferential pairing within genomes may be occurring in the amphidiploid.

Table 4.18. Embryo viability and uniformity of flowers of offspring from diploid and amphidiploid D. superbiens, D184 as a female parent.

Cross Number	Parentage	Embryo Viability (%)	Flower Shape, Size and Color
K1287	D184-2N X D. phalaenopsis, D354-4	59	Variable shape and size of flowers. Flower color ranged from light lavender to purple to lavender- bronze
K1291	D184-2N X D. phalaenopsis, 'Kosaki'	65	Variable flower size. Flower color ranged from light lavender to purple to dark purple
K1292	D184-2N X D. phalaenopsis, D390-60	41	Variable shape and size of flowers. Flower color ranged from light lavender to bronze-lavender to bronze-purple to lavender to purple
K1293	D184-4N X D. phalaenopsis D354-3	94	Uniform shape and size of flowers. Flower color uniform, dark purple
K1298	D184-2N X D. phalaenopsis, D354-3	50	Variable flower size. Flower color ranged from lavender to purple

Table 4.18. (Continued) Embryo viability and uniformity of flowers of offspring from diploid and amphidiploid D. superbiens, D184 as a female parent.

Cross Number	Parentage	Embryo Viability (%)	Flower Shape, Size and Color
K1319	D184-4N X D. phalaenopsis, D356-1	96	Uniform shape and size of flower. Flower color uniform dark purple.
K1320	D184-2N X D. phalaenopsis, D356-1	76	Variable flower shape and size. Flower color ranged from lavender to dark purple.

Table 4.19. Variability in flower measurements of D184 progeny. All measurements in cm.

Cross Number	Variable	Minimum	Maximum	Mean	Standard Deviation	CV (%)
K1287	Flower Length	4.0	6.0	5.0	0.7	13.72
	Flower Width	4.0	6.0	5.7	0.6	10.76
	Pedicel	2.0	3.0	3.2	0.3	9.96
	Petal Length	2.0	4.0	3.4	0.4	13.00
	Petal Width	1.0	3.0	2.3	0.5	22.38
	Dorsal Sepal Length	2.0	3.0	3.0	0.5	15.71
	Dorsal Sepal Width	1.0	2.0	1.3	0.4	32.09
K1291	Flower Length	4.0	6.0	5.7	0.6	10.36
	Flower Width	5.0	8.0	6.8	0.9	13.71
	Pedicel	3.0	4.0	3.6	0.4	10.71
	Petal Length	3.0	4.0	4.0	0.5	12.36
	Petal Width	2.0	3.0	2.6	0.3	11.00
	Dorsal Sepal Length	2.0	4.0	3.0	0.4	10.91
	Dorsal Sepal Width	1.0	1.0	1.3	0.2	13.32
K1292	Flower Length	4.0	5.0	4.9	0.4	9.02
	Flower Width	4.0	7.0	5.7	0.7	11.98
	Pedicel	3.0	4.0	3.8	0.4	11.29
	Petal Length	2.0	4.0	3.0	0.4	11.00
	Petal Width	1.0	2.0	2.4	0.2	9.73
	Dorsal Sepal Length	2.0	3.0	2.9	0.3	10.00
	Dorsal Sepal Width	1.0	1.0	1.2	0.1	8.45

Table 4.19 (Continued) Variability in flower measurements of D184 progeny. All measurements in cm.

Cross Number	Variable	Minimum	Maximum	Mean	Standard Deviation	CV (%)
K1293	Flower Length	4.0	5.0	4.9	0.3	6.97
	Flower Width	5.0	6.0	6.0	0.3	5.62
	Pedicel	2.0	3.0	3.2	0.3	8.59
	Petal Length	3.0	3.0	3.6	0.2	6.22
	Petal Width	2.0	3.0	2.8	0.3	7.04
	Dorsal Sepal Length	2.0	3.0	3.1	0.2	7.65
	Dorsal Sepal Width	1.0	1.0	1.5	0.2	10.33
K1298	Flower Width	3.0	6.0	5.4	0.6	11.76
	Flower Length	3.0	5.0	5.0	0.5	10.00
	Pedicel	2.0	4.0	3.0	0.6	17.82
	Petal Length	2.0	4.0	3.3	0.4	12.23
	Petal Width	1.0	2.0	2.0	0.2	10.00
	Dorsal Sepal Length	2.0	3.0	3.0	0.3	11.01
	Dorsal Sepal Width	0.0	1.0	1.2	0.2	16.73

Figure 4. Flower color variation in D. superbiens hybrids. Upper two rows: K1291 (D. superbiens 'D184 2N' x D. phalaenopsis 'Kosaki'). Lower two rows: K1293 (D. superbiens 'D184 4N' x D. phalaenopsis 'D354-3').

Thus, D184 4N would be a desirable parent for producing a seed-propagated cultivar because of its increased fertility and uniformity of offspring. Such a cross is called D. xLouis Bleriot and is presently grown for lei-flower production.

4.3 Androgenesis in D. xLouis Bleriot x D. xJaquelyn Thomas Amphidiploids

4.3.1 Chromosome Counts

Chromosome numbers of 209 offspring ranged from 38 to 96 (Table 4.20). The majority of the offspring were aneuploids in the 3-1/2N level (2n=65-68), derived from the fertilization of 1-1/2N eggs (n=ca.27 to 30) from the triploid parent and normally reduced gametes from the amphidiploid (n=2N=38). Unreduced gametes (n=3N=57) from the triploid and normally reduced gametes from the amphidiploid probably gave rise to pentaploid offspring (2n=95). Chromosome numbers from 58 to 73 and 94 to 96 are expected of 3N x 4N crosses (Kamemoto et al., 1972).

In ten out of the 11 crosses, offspring with chromosome numbers of 2n=38 or 2n=39 were recovered. All closely resembled the amphidiploid pollen parent, and were presumed to be of androgenetic origin (Figure 5). Twenty-three androgenetic offspring had the chromosome number of 2n=38, while seven had the

Table 4.20. Somatic chromosome numbers of offspring from crosses involving triploid D. xLouis Bleriot and D. xJaquelyn Thomas-type amphidiploids.

Cross	Somatic Chromosome Number									
	38	39	58-60	61-64	65-68	69-73	75-76	87-92	94-96	Total
K1056	5		2	7	17	1				32
K1057	2	1		1	9	3	1			17
K1058	1	1	1	6	5	4	1			19
K1059	5	1	1	3	12					22
K1060	2	1		5	5	2	1	2	1	19
K1061	3			1	9	2				15
K1062	1			3	5	2	1		2	14
K1063		3		2	11				1	17
K1064	3		1	5	9	2		1	1	22
K1065	1			5	2	5		1		14
K1066				3	3	4		5	3	18
Total	23	7	5	41	87	25	4	9	8	209

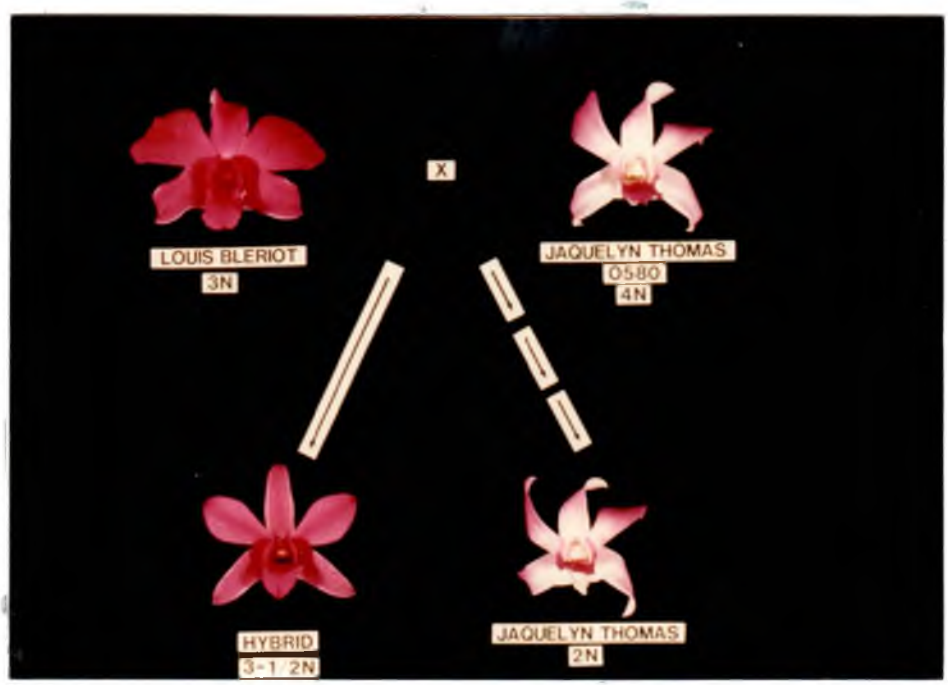

Figure 5. Hybrid offspring from K1057, a cross between triploid 'Louis Bleriot' and amphidiploid D. xJaquelyn Thomas '0580-4N' along with androgenetic offspring.

chromosome number of 2n=39, a trisomic number. The 2n=38 offspring are presumed to have originated from normally reduced haploid pollen from the amphidiploid parent, which entered the ovule, and then developed without syngamy. In the case of the trisomics, the functional pollen must have been n=39 (2N + 1), which could have resulted from a slight irregularity during microsporogenesis in the amphidiploid.

Two tetraploid seedlings with a somatic chromosome number of 2n=4N=76 were recovered from crosses K1057 and K1060. The flowers were slightly larger than those of the polyhaploids, but identical in shape and color to the male parents (Figure 6). These androgenetic tetraploid offspring may have arisen either from spontaneous doubling of the somatic chromosomes during the early development of an androgenetic embryo, or from unreduced pollen entering the ovule and developing without syngamy. Although two individuals from K1058 and K1062 had a chromosome number of 2n=75, these were not observed in flower, and hence, their origin was not determined.

The percentage of androgenetic offspring obtained based on the chromosome numbers of individuals counted was 15.3%, which is an exceptionally high number. If the percentage were based on the total number of

Figure 6. Diploid and amphidiploid androgenetic offspring of K1057 (D. xLouis Bleriot 'LBH' x D. xJaquelyn Thomas 'O580-4N') and K1060 (D. xLouis Bleriot 'LBK' x D. xJaquelyn Thomas 'Y166-1'). Top row from left to right: amphidiploid 'O580-4N', androgenetic amphidiploid and androgenetic diploid. Bottom row from left to right: 'Y166-1', androgenetic amphidiploid and androgenetic diploid.

seedlings grown in 2-inch pots for the 11 crosses and it is assumed there are no androgenetic offspring among the rest, the percentage of androgenetic offspring obtained was 4.5%, still a high number. Kostoff (1942) suggested that frequencies should be calculated on the basis of the number of available ovules, and not from the number of normal plants produced at the same time, as the elimination of hybrid embryos overestimates natural frequency. When androgenetic frequency is calculated for 10,000-15,000 ovules per seed pod, which is the average for Dendrobium (Sagawa and Israel, 1964), androgenetic frequency ranged from one per 1,666 progeny to one per 15,000 progeny (Table 4.21). This is relatively high compared to other crops where this phenomenon has been observed. In maize, androgenetic individuals occurred at a ratio of approximately one to every 80 monoploids of maternal origin, or one per 50,000 offspring (Chase, 1963).

Several factors may have contributed to the remarkably high occurrence of androgenesis in crosses involving D. xLouis Bleriot. Because the Louis Bleriot clones were triploid, fertility was low and a relatively small number of seeds were available in a seed pod. The majority of the hybrids were aneuploids between the triploid and tetraploid levels with

Table 4.21. Computed frequencies of androgenesis in crosses where androgenesis was observed.

Cross	Number of Androgenetic Offspring	Androgenetic Frequencies Based on	
		10,000 Ovules	15,000 Ovules
K1056	5	1/2,000	1/3,000
K1057	4	1/2,500	1/3,750
K1058	2	1/5,000	1/7,500
K1059	6	1/1,667	1/2,500
K1060	4	1/2,500	1/3,750
K1061	3	1/3,333	1/5,000
K1062	1	1/10,000	1/15,000
K1063	3	1/3,333	1/5,000
K1064	3	1/3,333	1/5,000
K1065	1	1/10,000	1/15,000

chromosome numbers ranging from 58 to 75, and many may not have been as vigorous as normal euploids. Thus the euploid androgenetic offspring undoubtedly had a selective advantage over the aneuploids at various stages of operations from germination, transflasking, compotting and planting into 2-inch pots.

4.3.2 Cymbidium Mosaic Virus Assay Results

Out of 30 androgenetic offspring assayed for Cymbidium Mosaic Virus (CyMV) with ELISA tests, 28 were shown to be negative and two were positive. The parents were heavily infected with the virus. The negative results obtained for the androgenetic offspring were similar to the earlier results of Yuen et al. (1979) on offspring from parents heavily infected with the virus. The two virus-positive offspring most likely became infected during cultivation, for the plants were assayed for virus after two years in cultivation. Most of the plants in the same greenhouse were infected with the virus and Okemura et al., (1984) have shown that infection of plants with CyMV increased linearly with time in cultivation.

More recently, Chia et al. (in press) detected 100% CyMV infection in seedlings. CyMV was assayed

using nuclear acid spot hybridization. This assay is more sensitive than ELISA and has been reported to detect virus concentrations at the picogram level. Thus it might be possible that although the androgenetic offspring tested negative for CyMV with ELISA tests, a more sensitive test may detect the virus at low concentrations.

The amphidiploid parents used in the present study are important breeding plants utilized in the production of seed-propagated commercial cut flower cultivars in Hawaii. However, they are infected with the virus, which affects growth and flower production (Sheehan et al., 1981). Those androgenetic offspring that tested negative for the virus have been mericloned and treated with colchicine in aseptic culture to induce chromosome doubling. The androgenetic or colchicine-doubled tetraploids should be identical or nearly-identical genetically to the amphidiploid parents, but without detection of CyMV with ELISA tests. These newly induced amphidiploids should be valuable for the production of the seed-propagated cultivars.

4.4 Conclusions

There were no differences among intersectional groupings in the morphological comparisons between the diploid and amphidiploid selections. Some, but not all selections had fewer racemes and fewer flowers per raceme in the amphidiploids. Most of the amphidiploids had larger flowers and increased size of flower segments. Amphidiploids are however, considered desirable because the lower yields are compensated by flower quality.

Sporad formation varied with different species combinations. Two Ceratobium-Eleutheroglossum selections had high frequencies of tetrads in the diploid form and lower frequencies of tetrads in the corresponding amphidiploids, suggesting relatively good homology of the parental genomes. However, a third Ceratobium-Eleutheroglossum selection exhibited a lower frequency of tetrads with a high dyad frequency in the diploid and higher tetrad frequency in the amphidiploid, leading to the conclusion that the parental genomes in this hybrid were not as closely related as those of the two other Ceratobium-Eleutheroglossum selections. The Phalaenanthe-Ceratobium amphidiploids all had a higher percentage of tetrads, except for D184 (D. superbiens), in which the

percentage of tetrads was high in both the diploid and amphidiploid. This suggests that there are different degrees of homology between Phalaenanthe and Ceratobium genomes also, and that in the amphidiploid there is preferential pairing of parental genomes. The Phalaenanthe-Eleutheroglossum selection had a high percentage of tetrads with microcytes in the amphidiploid, which suggests a high degree of homology between the Phalaenanthe and Eleutheroglossum genomes.

Test crosses with D. rumphianum and D. stratiotes showed a much higher success rate when the amphidiploids were used as females. Almost all crosses using the amphidiploids as pollen parents were unsuccessful, although functional gametes and fertility were expected in the amphidiploids from the high frequencies of tetrads. Therefore non-meiotic factors may have been responsible for male sterility.

The breeding behavior of four selections were evaluated for their suitability for the production of seed-propagated cultivars. The D. xJaquelyn Thomas amphidiploid 'K333-22' produced mostly variable offspring, unlike the earlier D. xJaquelyn Thomas amphidiploids successfully used in the production of seed-propagated cutflower cultivars. The variability in the offspring is presumably a result of a lack of

preferential pairing within the genomes, although the high percentage of tetrads formed suggests otherwise. The amphidiploid K333-22 was thus unsuitable for the production of seed-propagated cultivars. The amphidiploid D. xMemoria Edward Trevor 'D251' produced offspring which were not only variable but also did not possess desirable flower characteristics. Hence it was also determined to be unsuitable for producing seed-propagated cultivars. Although the offspring of amphidiploid D. xAutumn Lace 'K432-3' had uniform color, they had undesirable characteristics such as weak plant bases, susceptibility to phytophtora, or high bud drop. There were also slight variation observed in a cross with D. stratiotes, indicating that some multivalent associations may have occurred in the amphidiploid. The progenies of amphidiploid D. superbiens 'D184' crossed with D. phalaenopsis were uniform and therefore the amphidiploid can be used to produce a seed-propagated D. xLouis Bleriot, which is D. superbiens x D. phalaenopsis, and is a clonally propagated commercial cultivar.

Androgenesis was observed in 10 out of 11 crosses involving triploid D. xLouis Bleriot (D. superbiens x D. phalaenopsis) and four different D. xJaquelyn Thomas amphidiploids. The majority of the androgenetic

offspring had the diploid chromosome number of 2n=38. Seven androgenetic offspring had the aneuploid number of 2n=39 (=2n+1). Two androgenetic offspring from two different crosses were determined to be tetraploids with the chromosome number of 2n=4N=76. These results suggest that the amphidiploids had normal microsporogenesis resulting in the production of functional male gametes with the normal diploid number (n=2N=38). Occasional misdivisions may have occurred, possibly a univalent being included in the meiotic product, to result in gametes with the 2N+1 number. The occurrence of dyads in the amphidiploid as well as restitution gametes with the 4N number must be very rare, as only one tetraploid androgenetic offspring was recovered in each of the two crosses were tetraploid offspring where encountered.

LITERATURE CITED

Bobisud, C. and H. Kamemoto. 1982. Selection and inbreeding in amphidiploid Dendrobium (Orchidaceae). J. Amer. Soc. Hort. Sci. 107:1024-1027.

Burk, L.G. 1962. Haploids in genetically marked progenies of tobacco. J. Heredity. 53: 222-225.

Campos, F.F. and D.T. Morgan,Jr. 1958. Haploid pepper from a sperm. J. Heredity. 49:134-137.

Chaicharoen, S. and K. Sajaew. 1981. Autopolyploidy in Dendrobium phalaenopsis. J. Sci. Soc. Thailand 7:25-32.

Chase, S.S. 1963. Androgenesis - its use for transfer maize cytoplasm. J. Heredity 54:152-158.

Chia, T.F., Y.S. Chan and N.H. Chua. In press. Large scale screening of cymbidium mosaic and odontoglossum ringspot viruses in cultivated orchids by nucleic acid spot hybridization. Proc. 13th World Orchid Conf.

Cribb, P.J. 1986. A revision of Dendrobium sect. Spatulata (Orchidaceae). Kew Bull. 41: 615-692.

Dockrill, A.W. 1969. Australian Indigenous Orchids Vol. 1. The Society for Growing Australian Plants, Sydney. Pp.457-460.

Goodsell, S.F. 1961. Male sterility in corn by androgenesis. Crop Science 1: 227-228.

Greisbach, R. 1985. Polyploidy in Phalaenopsis improvement. J. Heredity. 76:74-75.

Hagerup, O. 1947. The spontaneous formation of haploid, polyploid and aneuploid embryos in some orchids. K. Dansk. Videnskab. Selskab. Biol. Meddelel. 20:1-22.

Horn, W. 1977. Parthenogenetic and androgenetic offspring from interspecific crosses in Begonia. Proc. 8th EUCARPIA Congress. Pp.351-355.

Jackson, R.C. 1976. Evolution and systematic significance of polyploidy. Ann. Rev. Ecol. Syst. 7:209-234.

Kam, L.N.W. and H. Kamemoto. 1980. Diploid and tetraploid Aranda Wendy Scott from meristem culture. Na Okika o Hawaii 9(2): 7-12.

Kamemoto, H. 1959. The origin and significance of polyploidy in Vanda. Pacific Orchid Soc. Bull. 16:77-95.

Kamemoto, H. 1980. Breeding Dendrobiums for commercial cut-flower production. Proc 3rd ASEAN Orchid Congress. Pp.96-110.

Kamemoto, H. 1984. Recent research on breeding dendrobiums. Proc. Asian Pacific Orchid Congress. Pp.29-33.

Kamemoto, H. 1985. Seed-propagated amphidiploid dendrobium cultivars. HortScience 20:2, 163.

Kamemoto, H. and J. McConnell. 1984. Breeding with amphidiploid dendrobiums. Proc. Eleventh World Orchid Conf. Pp.220-223.

Kamemoto, H. and M. Tara. 1968. Chromosome inheritance in reciprocal crosses of Dendrobium phalaenopsis 'Lyon's Light No. 1'. Proc. Amer. Soc. Hort. Sci. 92: 665-671.

Kamemoto, H. and G.J. Wilfret. 1980. Inter- and multi-sectional Dendrobium hybrids. Proc. 9th World Orchid Conf. Pp.255-261.

Kamemoto, H., K. Shindo and K. Kosaki. 1964. Chromosome homology in the Ceratobium, Phalaenanthe, and Latourea section of the genus Dendrobium. Pacific Science 18:104-115.

Kamemoto, H., M. Takeshita and W. Meeyot. 1972. Breeding behavior of polyploid Dendrobium orchids. J. Amer. Soc. Hort. Sci. 97: 3-5.

Kosaki, K. and H. Kamemoto. 1961. Chromosomes of some Dendrobium species and hybrids. Na Pua Okika o Hawaii Nei 11: 75-86.

Kostoff, D. 1942. The problem of haploidy. Bibl. Genet. 13: 1-148.

Lanaud, C. 1988. Origin of haploids and semigamy in Theobroma cacao L. Euphytica 38:221-228.

Lee, Y.H. and H. Kamemoto. 1984. Cytology and breeding behaviour of diploid and amphidiploid Aranda Wendy Scott (Orchidaceae). Proc. 5th ASEAN Orchid Cong. Sem. Pp.89-91.

Leonhardt, K.W. 1987. Polyploidy influences floral morphology and vegetative characteristics of a Cymbidium species and two Cymbidium hybrids. Proc. 1987 Commercial Orchid Growers Conf. Pp.99-101.

MacLeod, R.A. 1947. Some effects of colchicine in orchids. Amer. Orchid Soc. Bull. 16:336-337.

McConnell, J. 1984. Breeding behavior of some amphidiploid Dendrobium. PhD. Dissertation, University of Hawaii, Honolulu, 112p.

Mehlquist, G. 1949. The importance of chromosome numbers in orchid breeding. Amer. Orchid Soc. Bull. 18:284-293.

Menninger, E. 1963. Diary of a colchicine-induced tetraploid cymbidium. Amer. Orchid Soc. Bull. 32:885-887.

Nakasone, H. and H. Kamemoto. 1961. Artificial induction of polyploidy in orchids by the use of colchicine. Hawaii Agr. Exp. Sta. Tech. Bull.

Niimoto, D.H. and Y.Sagawa. 1961. Ovule development in Dendrobium. Amer. Orchid Soc. Bull. 30:813-819.

Okemura, A. K., H. Kamemoto and M. Ishii. 1984. Incidence and expression of cymbidium mosaic virus in Dendrobium orchids. Hawaii Inst. of Trop. Agr. and Human Resources Res. Ser. 033.

Pandey, K.K. 1973. Theory and practice of induced androgenesis. New Phytol. 72:1129-1140.

Rieger, R., A. Michaelis and M. Green. 1976. Glossary of genetics and cytogenetics: classical and molecular (4th ed.). New York, Springer-Verlag.

Rotor, G.B. Jr. 1958. Colchicine as a tool in orchid hybridization. Proc. 2nd World Orchid Conf. Pp. 159-170.

Sagawa, Y. and H. Israel. 1964. Post-pollination ovule development in Dendrobium orchids. I. Introduction. Caryologia 17: 53-64.

Sagawa, Y. and H.L.Valmayor. 1966. Embryo culture of orchids. Proc. 5th World Orchid Conf. Pp. 90-101

Sanguthai, O., S. Sanguthai and H. Kamemoto. 1973. Chromosome doubling of a dendrobium hybrid with

colchicine in meristem culture. Na Okika o Hawaii 2:12-16.

Sanguthai, S. and Y. Sagawa. 1973. Induction of polyploidy in Vanda by colchicine treatment. Na Okika o Hawaii 2:17-19.

Schlecter, R. 1982. The Orchidaceae of German New Guinea, translation of the German text from Die Orchidaceen von Deutsch-Neu-Guinea. Translated by R.S. Rogers, H.J. Katz and J.T. Simmons. The Australian Orchid Foundation, Melbourne. Pp. 508-640.

Sheehan, T.J., G.C. Wisler and F.W. Zettler. 1981. Orchid viruses - their distribution and new detection techniques. Proc. 10th World Orchid Conf. Pp.279-285.

Stebbins, G.L. 1947. Types of polyploids: their classification and significance. Adv. Genet. 1:403-429.

Storey, W.B. 1956. Diploid and polyploid gamete formation in orchids. Proc. Amer. Soc. Hort. Sci. 68:491-502.

Tanaka, R. and H. Kamemoto. 1960. Meiotic chromosome behavior in diploid and polyploid Vanda orchid hybrids. Cytologia 25:405-418.

Tanaka, R. and H. Kamemoto. 1984. Chromosomes in orchids: counting and numbers. In: Arditti, J. (ed.). Orchid biology - review and perspectives, III. Cornell Univ. Press. Pp. 323-410.

Watrous, S.B. and D.E. Wimber. 1988. Artificial induction of polyploidy in Paphiopedilum. Lindleyana 3:177-183.

Wilfret, G.J., T. Takeshita and H. Kamemoto. 1979. Genome and karyotype relationships in Dendrobium

(Orchidaceae). III. Meiotic behavior. J. Amer. Soc. Hort. Sci. 104:43-46.

Wimber, D.E. and A. Van Cott. 1967. Artificially induced polyploidy in Cymbidiums. Proc. 5th World Orchid Conf. pp. 27-32.

Wimber, D.E. and D.R. Wimber. 1968. Floral characteristics of diploid and neo-tetraploid Cymbidiums. Amer. Orchid Soc. Bull. 37:572-576.

Yuen, C.K.K.H., H. Kamemoto and M. Ishii. 1979. Transmission of Cymbidium mosaic virus through seed propagation in Dendrobium. Amer. Orchid Soc. Bull. 488(12):1245-1247.